


Phosphorus retention in constructed wetlands enhanced by zeolite- and clinopyroxene-dominated lava sand

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Abstract

Constructed wetlands (CWs) are engineered systems for treating wastewater by sequestering nutrients and contaminants. Our aim was to assess the main phosphorus (P) binding states in operating CWs to assess P saturation and indications on P recycling potential of filter materials, which might be necessary under future peak P scenarios. The investigated vertical flow CWs (operation time up to 16 years) are based on either fluvatile (Fluv) sand or zeolite- (Ze-LS) and clinopyroxene (Cl-LS)-dominated lava sand. Organic and inorganic P accumulated in all CWs independent of filter materials and showed a considerable increase with operation time. Concentrations of P decreased sharply with depth in the Fluv-CWs compared to only a slight decrease in the lava sand CWs, with P concentrations of deeper horizons approximating the relatively P enriched original lava sand substrates. Orthophosphate was the dominant pool in all CWs, while the sum of organic fractions ranged between 11% and 33%. Sequential extraction indicated that P was mainly associated with Fe and Al (hydr)oxides for Fluv-CWs and Ze-LS-CWs, while Ca and Mg bound mineral phosphates dominated in Cl-LS-CWs. Oxalate extractions pointed to a clear dominance of P fractions associated with poorly crystalline Fe- and Al-(oxy)hydroxides. Solution ³¹P NMR analyses revealed that inositol hexakisphosphates were a major pool of organic P in surface layers of CWs, which increased with operation time. With a maximum of 0.5% P content, filter sands do not appear to be a suitable fertilizer for direct application to agricultural fields. The dominance of inorganic, poorly crystalline P species point to potentially high desorption capacity which might be investigated further, to assess recycling potential of P or usage of filter materials as soil amendments with relatively high plant available P. The latter might become feasible and economically attractive under future P scarcity. Simultaneously, P saturation indexes (DPS) did not indicate an imminent P saturation of filters, since P accumulation was not restricted by binding to Al and Fe minerals.

Christine Alewell and Jen-How Huang shared first authorship.

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KEYWORDS

filter materials, NMR spectroscopy, organic P species, P recycling, P saturation, sequential P extraction, vegetated soil filters, waste water cleaning

1 | INTRODUCTION

Constructed wetlands (CWs) are engineered systems that use the naturally occurring functions of vegetation, soils and microorganisms to treat wastewater (Wu et al., 2018). For decades, CWs have been applied for the removal of metals (Guittonny-Philippe et al., 2014; Huang et al., 2017), organic pollutants (Zhang et al., 2018), and macronutrients such as nitrogen and phosphorus (P) from domestic and industrial wastewaters and agricultural runoffs (Bruch et al., 2011b; Bruch et al., 2014; Huang et al., 2017; Vohla et al., 2011; Vymazal, 2007). Whilst P is often a limiting macronutrient for living organisms in aquatic and terrestrial ecosystems (Paytan & McLaughlin, 2007; Soht et al., 2017), especially in the context of agricultural production (Alewell et al., 2020), urbanization and intensification of agriculture have caused a P enrichment of surface aquatic ecosystems, which has often led to hypoxia, eutrophication (Withers & Jarvie, 2008), and a loss of biodiversity in fresh- and ocean waters (Davis et al., 2019; Lind et al., 2019; Pant, 2020).

Phosphorus in wetlands may be present in dissolved and suspended forms, sorbed to organic and mineral particles, or contained within organic molecules, humic substances and biomass (Ballantine & Tanner, 2010; Kuusemets & Lohmus, 2005; Paludan & Jensen, 1995; Vera et al., 2014). The retention of P in wetlands is considered to be largely through plant uptake, microbial assimilation, adsorption and precipitation processes with soil constituents (Ballantine & Tanner, 2010; Vohla et al., 2011). Adsorption and precipitation processes tend to dominate in hypereutrophic systems due to biological saturation and the strong affinity of P to minerals such as iron (Fe) and aluminium (Al) (hydr)oxides in acidic soils, and calcium (Ca) and magnesium (Mg) in alkaline soils (Karjalainen et al., 2016).

The long-term removal capacity of P in CW systems requires the selection of filter materials that promote P retention, which is largely based on their P adsorption or retention capacity (Ballantine & Tanner, 2010). These can include: allophane, Papakai tephra, tree bark, limestone, alum, slag, seashells, shell-sand, or a mixture of subsoil and topsoil (Ballantine & Tanner, 2010). However, fluvial sand (Fluv) is the most commonly used filter material in CWs due to its high availability and low cost (Bruch et al., 2014). Nevertheless, the P sorption capacity of filter materials may not always explain the overall P removal efficiency in CWs. Cui et al. (2008) highlighted the different P adsorption capacities and P removal efficiencies of nine different filter materials, which appear to be related to their physicochemical properties, for example, pH, redox potential (Eh), Ca, Fe and Al contents. In addition, the mineral composition of the filter materials may also affect the element removal efficiency of wastewaters. For example, filter materials containing zeolites can increase the metal sorption

capacity and also have a large hydraulic regulation function due to their swelling capacity (Bruch et al., 2011b; Bruch et al., 2014). This prolongs the retention time of the wastewater in the filter matrix and thus the equilibration time of substrates at the filter solid-water interface, generally resulting in a high removal efficiency of metals (Huang et al., 2017) and nutrients (Bruch et al., 2014). For substances with strong adsorption affinity to Fe(hydr)oxide such as P, the Fe(hydr)oxides containing filter materials may change from sorption to desorption substrates depending on oxygen saturation (Laakso et al., 2016), that is, Fe(hydr)oxide dissolution and P release during the anoxic cycle and fixation in the oxic period. Therefore, a detailed understanding of both the P retention capacity and mobility along the CW profiles as a function of filter material is needed when designing CWs.

There is limited information on the chemical nature of P that accumulates in filter materials of CWs. Understanding the dominant P pools and the mechanisms responsible for their accumulation or release will help improve the construction of CWs for increased P retention capacity. However, beyond striving for maximum P sorption capacities, an increased understanding of P species is also required to develop strategies that release P for recycling. Previous studies on CWs using sequential P extraction have indicated the prevalent association of P with Fe(hydr)oxides (Paludan & Jensen, 1995). Furthermore, studies using solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy on NaOH-EDTA extracts have revealed that CWs contain relatively high concentrations of both inorganic P (Pi) and organic P (Po) (Turner et al., 2006a; Turner et al., 2006b). The chemical nature of the latter was largely attributed to that of DNA, and the alkaline hydrolysis products of phospholipids and RNA, which exhibit NMR signals in the phosphomonoester region (Turner et al., 2006a). However, the chemical nature of a large portion of the organic P remained unclear. New information on the chemical nature of these pools might provide new insight on the mechanisms governing P sequestration, stability and turnover in these systems.

CWs are highly important to remediate P in municipal wastewater. Thus, this study aimed to (a) quantify the impact of two common filter materials (fluvial and lava sands) on P retention mechanism along depth profiles in operating CWs, (b) understand the binding state and chemical nature of P in CWs, (c) elucidating the potential role of different mineral components to the P retention in different filter materials, and (d) assess the likelihood of potential recycling of P from filter materials. We selected nine CWs in Germany containing fluvial or lava sand as filter material. All facilities exhibited high removal rates of chemical oxygen demand (COD), nitrogen (especially ammonia) and P, which makes them suitable wastewater facilities for local authorities (Bruch et al., 2014; Moradpour & Mayer, 2012).

2 | MATERIAL AND METHODS

2.1 | Site description

All sampled CWs were located within 100 km from each other in southwestern Germany (Huang et al., 2017), and are used as municipal plants to treat wastewater using a vertical flow regime. All CWs consisted of a gravel layer at the bottom of the profile (25–30 cm thick, 4–8 mm grain size), and an overlying filter layer (~70 cm thick). Three different filter materials were used and have been under operation for varying lengths of time at the time of sampling, these include: fluvial sands (Fluv) in Schneebergerhof (Fluv-16yr), St. Alban (Fluv-9yr) and Würzweiler (Fluv-12yr and Fluv-8yr), clinopyroxene-dominated lava sand (CI-LS) in Medelsheim (CI-LS-6yr) and Tettingen-Butzdorf (CI-LS-7yr), and zeolite-dominated lava sand (Ze-LS) in Büschdorf (Ze-LS-8yr) and Riesweiler (Ze-LS-6yr).

X-ray diffraction (XRD) analysis (AXS D8 XRD, Bruker, Karlsruhe, Germany,) showed that the Fluv material was dominated by quartz, with minor amounts of feldspar (albite, microcline) and clay minerals (kaolinite, illite, chlorite and vermiculite [Huang et al., 2017]). The CI-LS material contained clinopyroxene, with minor amounts of olivine (forsterite), feldspathoid (leucite) and chabazite (Huang et al., 2017). The Ze-LS was dominated by phillipsite and chabazite, but there was also some clinopyroxene, olivine (forsterite), feldspathoid (nepheline), magnetite and quartz present. This suggests the Ze-LS material contains lava sand that has been mixed with some quartz sand. The specific surface area of the Fluv, CI-LS and Ze-LS materials was ~1, 18 and 72–80 m² g⁻¹, respectively, as determined by BET N₂ analysis (Bruch et al., 2014). All CWs received wastewater from the nearby municipalities, which was supplied onto the filter materials intermittently. This involved 1 week of loading followed by 1 week of resting, during which time the filters become dry and oxic.

The facilities are connected to a separated (in Fluv-8 years and CI-LS-6yr) or mixed (all other CWs) sewer system, designed with an surface area of between 2.4 and 5 m² per population equivalent and having operating times between 6 and 16 years (Huang et al., 2017). All CWs are likely to have comparable loads of annual wastewater input based on their similar municipal environments (Hasselbach, 2013). Substrate input appears to remain relatively constant as shown by the small variation in total organic carbon, total P and NH₄-N of the inflow at Ze-LS-6yr from 2007 to 2011 (Bruch et al., 2011a).

All CWs were originally planted with reed (*Phragmites australis*) with a density of 4 plants per m², but the reed has become partly displaced with stinging nettle (*Urtica dioica*) over time in some areas.

All CWs were reported to have removal rates of 80%–95% in COD, 90%–100% ammonia and 55%–80% P (Bruch et al., 2011b). Hasselbach (2013) investigated the P concentrations in the inlet and outlet waters at the Ze-LS CWs re-investigated in this study and found an overall P removal efficiency of 46%–83%.

Additional information on the CWs and their operating conditions can be found in Huang et al. (2017), Bruch et al. (2011b), Hasselbach (2013) and Bruch et al. (2014).

2.2 | Sampling

In June 2012, filter material was sampled in triplicate at each CW with a soil corer (diameter of 6 cm) to a depth of 60 cm (with the exception of the Ze-LS-6yr site, which was water saturated in the deeper layers). Sampling points were well distributed within the area of the CW but at least with 1 m distance from the edge. Each core sample was sliced into six vertical sections of 10 cm. All samples were dried at 40°C, passed through a 5 mm sieve, and then milled prior to chemical analyses. The bulk densities of Fluv sand, Ze-LS and CI-LS were ~1.4 g cm⁻³.

2.3 | Analysis of phosphorus concentrations

Phosphorus concentrations were measured in the original substrates of Ze-LS, which can be considered as baseline values for estimating the P accumulation rate at these sites. Since no original substrate was available for the CI-LS as well as for the Fluv, P concentrations in the deepest layer (depth of 50–60 cm) at the Fluv sites were analysed and considered to represent baseline values. The net accumulation rate of P was calculated by taking the difference in their concentration for each layer and that of the original substrates, and dividing them by the operation time at each CW.

Total P concentrations were determined at all CWs and layers, whereas pH was measured in three layers (0–10, 20–30 and 40–50 cm). Sequential P extraction and oxalate extraction was carried out at all CWs and in two layers (0–10 and 40–50 cm, or 20–30 cm for Ze-LS-6yr for which it was the deepest available layer). The NaOH-EDTA extraction technique for solution ³¹P NMR analysis was carried out on one replicate of each CWs in the 0–10 cm layer.

Sequential P extraction was carried out on 0.2 g of filter material using a modified scheme based on the methods of Tiessen and Moir (1993) and Bowman and Moir (1993). The procedure included the following steps:

- (1) Resin strip (6 × 2 cm), 10 ml water, at 20°C for 18 h, followed by rinsing with 5 ml water and elution of the resin with 30 ml 0.1 M NaCl, representing the readily soluble P fraction; P in the total of 15 ml water remaining after removal and rinsing of the resin strip representing a second readily soluble fraction;
- (2) 10 ml of 0.25 M NaOH and 0.05 M EDTA, at 20°C for 18 h, representing organic P, Al and Fe mineral phosphates, and P more readily sorbed to Fe and Al (hydr)oxides;
- (3) 10 ml 1 M HCl, at 20°C for 18 h, representing the Ca and Mg mineral phosphates; and
- (4) Residual P was taken as the difference between the sum of the aforementioned steps and the total concentration of filter material P as determined by digestion with H₂SO₄ and H₂O₂ at 360°C (Anderson & Ingram, 1993).

The first resin eluate was analysed only for molybdate-reactive P using malachite green (Ohno & Zibilske, 1991), from here on referred to

as inorganic P (Pi). All other supernatants were analysed colorimetrically for Pi using malachite green (Ohno & Zibilske, 1991) and for total P using inductively coupled plasma-optical emissions spectroscopy (ICP-OES). The difference between the concentration of total P and Pi is that of molybdate-unreactive P, and is here on referred to as organic P (Po). Furthermore, we refer hereafter to the P eluted from the resin strip as readily available P, P in the supernatant of the resin extraction as water extractable P, the NaOH-EDTA extractable P as Al-Fe bound phosphates, and the HCl extractable P as Ca and Mg mineral phosphates.

2.4 | Extractable metals and phosphorus saturation index

Extractable metals were determined using the oxalate extraction technique of Schoumans (2000). Briefly, 2.0 g of filter material was extracted with 50 ml of 0.1 M oxalic acid and 0.175 M ammonium oxalate (pH 3.0) mixture and shaken in the dark for 4 h. The extracts were centrifuged at 4000 rpm for 10 min, filtered through a Whatman no. 40 filter paper, which was then acidified by mixing with 0.01 M HCl in a 1:4 ratio. The acidified filtrate was subsequently analysed for total P, Al and Fe using ICP-OES. Estimation of P saturation index of different filter materials was carried out as described by Nair et al. (2004). The degree of P saturation (DPS) ratio was calculated using Equation (1):

$$\text{DPS} = \frac{[\text{Oxalate extractable P}]}{0.5 \times ([\text{Oxalate extractable Al}] + [\text{Oxalate extractable Fe}])}. \quad (1)$$

2.5 | Extraction of organic phosphorus and preparation for NMR analysis

Concentrations of Po were determined using the method of Cade-Menun and Preston (1996). Briefly, 2 g of filter material was extracted with 40 ml of 0.25 M NaOH + 0.05 M Na₂-EDTA solution on a horizontal shaker at 160 rpm for 16 h. Extracts were centrifuged at 5000 rpm for 20 min, and the supernatant passed through a Whatman no. 42 filter paper. A 20 ml aliquot of the filtrate was frozen at -80°C, and then lyophilized and weighed (Doolette et al., 2010). This resulted in 521–644 mg of lyophilized material across all filter materials.

Preparation of lyophilized material for solution ³¹P NMR analysis is the same as that described in Reusser et al. (2020).

2.6 | Solution phosphorus-31 NMR spectroscopy

All spectra were obtained with a Bruker Avance III HD 500 NMR spectrometer equipped with a Bruker CryoProbe™ Prodigy probe (Bruker Corporation; Billerica, MA) at the NMR facility of the Laboratory of Inorganic Chemistry (Hönggerberg, ETH Zürich). The NMR

spectrometer was set at a ³¹P frequency of 202.5 MHz with inverse gated broadband proton decoupling and a 90° pulse of 12 μs duration. Solution ³¹P NMR spectroscopy was carried out on the filter materials with a total acquisition time ranging from 14 to 32 h with an average value of 25 h. Further details can be found in the Supporting Information.

All spectra were initially processed within TopSpin® software (Bruker Corporation, Billerica, MA). The spectra were then phased and baseline corrected, and defined integral ranges were established according to the presence of ³¹P NMR signal. The main integral regions can be found in the Supporting Information. Due to the presence of several overlapping NMR signals, spectral deconvolution fitting was carried out for the phosphonate region at δ 17.26–16.11 ppm and the combined orthophosphate and phosphomonoester region at δ 6.26–2.76 ppm. Spectral deconvolution fitting was carried out in these regions using Matlab (The MathWorks Inc., Natick, MA), as described in Reusser et al. (2020). Further details on the partitioning of these regions, peak assignments, and quantification of P in NaOH-EDTA using NMR can be found in the Supporting Information.

2.7 | Graphics and statistical analyses

All NMR graphics were carried out using R 3.2.0 (R_Core_Team, 2016).

3 | RESULTS AND DISCUSSION

3.1 | Phosphorus distribution along the CW profiles

Independent of the filter materials of the different CWs, total P was enriched in all surface layers (Figure 1), which reflects the vertical flow regime and limited downwards transport of P (Olila et al., 1997). In addition to the filter materials' strong binding affinity of P, the accumulation of P in the surface layer may have increased due to decreasing pH values. While original substrates had pH values of 8.5 (Huber, 2012), filter materials increasingly acidified in the surface horizons with pH 6–7 compared to pH 7.0–8.0 in the deeper horizons (Table S1 of the Supporting information). This is likely due to acidifying processes like mineralization and nitrification in spite of the input of relatively high pH sewage water on the surface. However, the adsorption capacity and affinity of P compounds to adsorbing surfaces generally increases with decreasing pH (Lü et al., 2017).

Concentrations of P in the upper layers of each CW were considerably greater than those in the corresponding baseline materials, and decreased more drastically with depth in the Fluv CWs than in the Cl-LS and Ze-LS CWs (Figure 1). The accumulation rate of P was strongest in the surface layer with a rate of ~200 mg P kg⁻¹ year⁻¹ in most CWs (Table S2 of the Supporting Information), reflecting the similar operating conditions among CWs (Huang et al., 2017). Higher

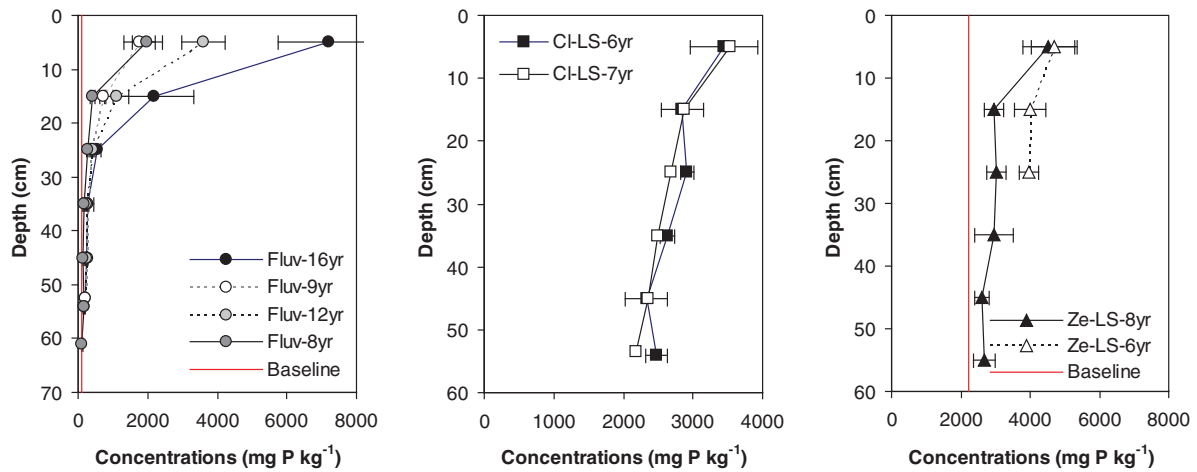


FIGURE 1 Concentrations of total phosphorus at different depths of fluviatile sand (Fluv) and clinopyroxene- (CI-LS) and zeolite-dominated lava sand (Ze-LS)-based constructed wetland (CW) profiles of different operation time. Red reference lines show the baseline concentrations of phosphorus in the starting materials of Ze-LS and in the deepest layers of fluviatile sand based CWs, respectively

accumulation rates of P in the surface layers at Fluv-16yr might at least partially be due to longer operation times; however, the high accumulations in the surface of Ze-LS-6yr (423 and 410 mg kg⁻¹ year⁻¹, respectively) imply different or additional mechanisms governing the accumulation of P at these sites. At 20–40 cm depth, P concentration was higher in CI-LS and Ze-LS CWs than in Fluv CWs, reflecting the higher baseline concentrations in these lava sand containing CWs. The latter might be due to the higher presence of metals (Huang et al., 2017), which have a strong binding affinity with P. In a previous study, we showed a high removal efficiency of metals in Ze-LS CWs (Huang et al., 2017). Zeolites not only increased the metal sorption capacity but they also adsorb large amounts of water, prolonging the equilibration time of metals at the filter solid-water interface (Bruch et al., 2011b; Bruch et al., 2014).

3.2 | Phosphorus sequential extraction in the filter materials of CWs

The greatest difference in the P composition of filter materials was observed in the surface layer, especially between the four Fluv CWs (Figure 2a). While surface layers of Fluv CWs and Ze-LS CWs were generally dominated by Fe and Al bound Pi, the majority of surface layer P of CI-LS CWs was Ca- and Mg-bound Pi.

In general, orthophosphate was a major P pool in all CWs and considerably increased over time (932 mg kg⁻¹ in the 8 years Fluv-Wü compared to 5148 mg kg⁻¹ in the 16 years Flu-Sc (Table 1), with Po comprising between 21% and 33% of total P in all CWs. Even though the sum of Po was also increasing from 261 mg kg⁻¹ in the 8 years Fluv-Wü compared to 756 mg kg⁻¹ in the 16 years Flu-Sc (Table 1), the relative proportion of Po decreased from 33% to 21% of total P in this chronosequence.

Pools of Al and Fe bound P in the surface layer had the largest increase compared to that in the 40–50 cm layer across all CWs (Figure 3d,e), followed by readily available and water extractable Pi (Figure 3a–c). Water extractable Pi and Al and Fe bound Pi and Po had significantly larger concentrations in the surface layer than at 40–50 cm depth in CI-LS and Ze-LS CWs (1.8–4.8 times). There were strong and significant correlations between the operation time of all CWs and the changes in concentrations in the 0–10 cm layer compared to the 40–50 cm layer for Al and Fe bound Pi ($r = 0.92$, $p < 0.01$, Figure 3) and Al and Fe bound Po ($r = 0.96$, $p < 0.01$, Figure 3e). The reason for this is likely the association of P with Fe and Al (hydr)oxides as the major P retention mechanism in CWs, independent of the filter materials. In soils and sediments, sorption to Al and Fe (hydr)oxides, complexation and co-precipitation with Al³⁺ and Fe³⁺ are the main abiotic processes governing P accumulation (Lü et al., 2017; Xu et al., 2006).

In the 40–50 cm layer at the Ze-LS CWs, Al and Fe bound Pi comprised 60% of the total P, whereas Ca and Mg mineral phosphates were dominant at CI-LS (50–60% of total P) (Figure 2c). In the 40–50 cm layer at the Fluv CW sites, both Al and Fe bound Pi and Ca and Mg mineral phosphates were the predominant fractions. These results indicate a different P composition of the filter materials among Fluv, CI-LS and Ze-LS. Surprisingly, significantly elevated concentrations of Ca and Mg mineral phosphates and/or residual P fractions were found in the surface layers of older Fluv-CWs (i.e., Fluv-16yr and Fluv-12yr CWs).

Results of the phosphorus sequential extraction indicated that organic P (Po) does not contribute more than 20% in most horizons of all three filter materials, in some exceptions up to 40% of total P (Figure 2). In principle, there are two major binding mechanisms to consider. While organic matter contains P and thus is a P storage in itself, organic matter also has a sorption capacity for P, which is likely via metal

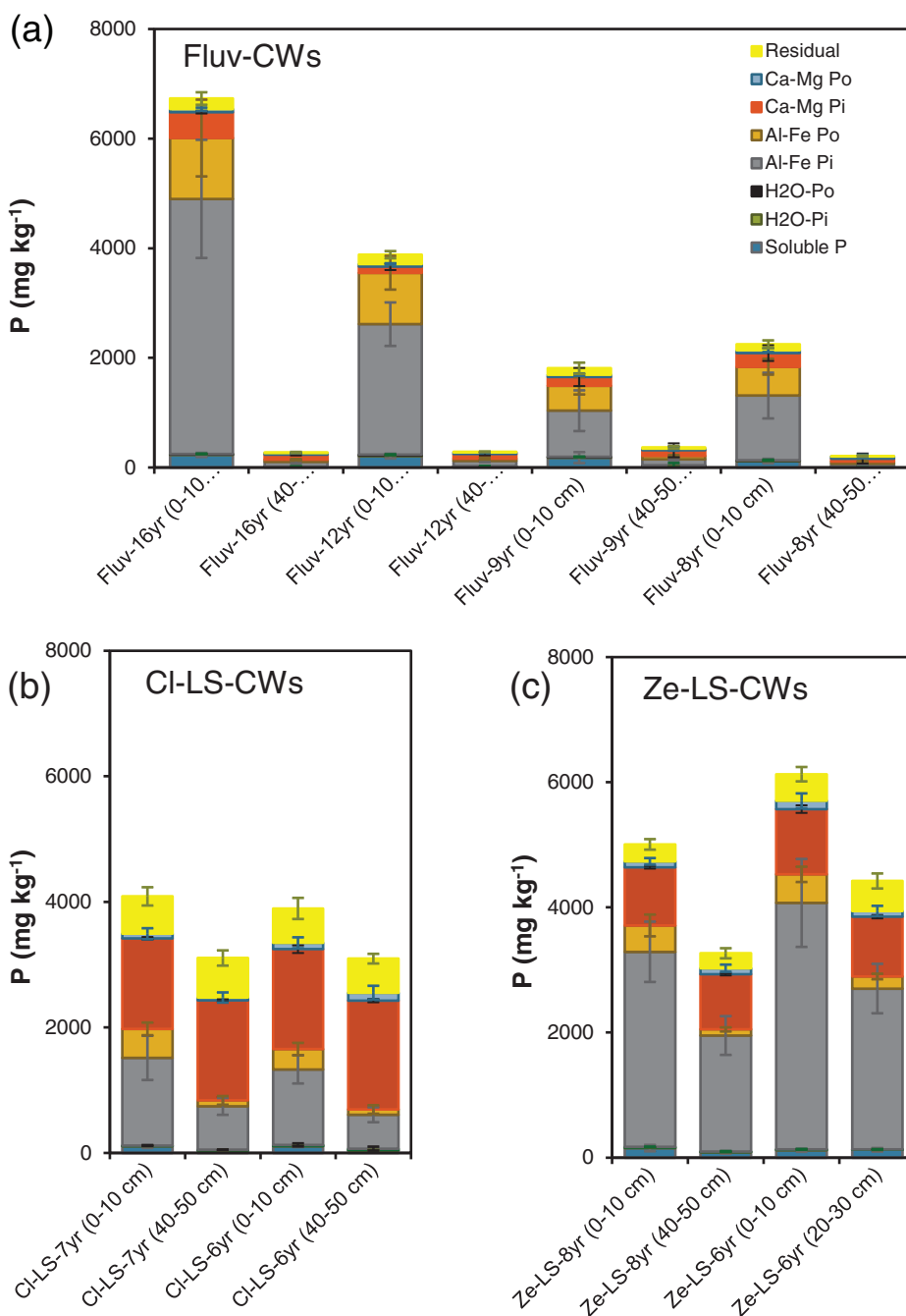


FIGURE 2 Phosphorus concentrations of sequential extraction-based fractions at 0–10 and 40–50 cm depths (20–30 cm depth at constructed wetland [CW] Ze-LS-6 years) of CW profiles based on (a) fluviatile sand (Fluv), (b) clinopyroxene-dominated lava sand (Cl-LS) and (c) zeolite-dominated lava sand (Ze-LS). Mean values and standard deviations of three spatial replicates at each site are shown. Ca-Mg Po and Pi = HCl extractable Ca- and Mg-bound organic and inorganic P, respectively. Al-Fe Po and Pi = NaOH-EDTA extractable Al- and Fe-bound organic and inorganic P. H₂O and resin-P refer to the water and readily soluble P fractions, respectively

bridges forming humic-metal-P complexes (Gerke, 2010). The general consensus is that iron and aluminium sesquioxides are the predominant P sorption sites, which is mirrored by our data (Figure 2). Simultaneously, organic matter might decrease the soil's P sorption capacity via competitive sorption reactions with DOC (e.g., Guppy et al., 2005). Thus, while some studies report no or even a negative relationship between P sorption and organic matter (Daly et al., 2001; Singh & Gilkes, 1991), other studies have reported significant positive relationships (Reddy et al., 1995). Since our data indicate that the P sorption sites of the filter material are not yet saturated, the role of organic matter in this respect is most likely not a regulating function at the moment.

3.3 | Chemical nature of organic phosphorus in NaOH-EDTA extracts of filter materials

Solution ³¹P NMR spectroscopy on NaOH-EDTA extracts was used to determine the chemical nature of Po in the 0–10 cm layer at each CW and with increasing time. Concentrations of P in NaOH-EDTA extracts of filter materials followed a similar trend to that measured in the aforementioned NaOH-EDTA extracts of the sequential chemical fractionation procedure (Table 1). Solution ³¹P NMR spectroscopy on NaOH-EDTA extracts revealed the presence of a multitude of P species (Figure 4, and Figures S1 and S2 of the Supporting Information).

TABLE 1 Concentrations (mg kg⁻¹) of phosphorus (P) species in NaOH-EDTA extracts of three different filter materials and up to four sampling times as determined from solution ³¹P nuclear magnetic resonance (NMR) spectroscopy

P species	Fluviatile				CI-LS		Ze-LS	
	Sc 16yr (1997)	Wü 12yr (2001)	St A 9yr (2004)	Wü 2 8yr (2005)	Te-1 7yr (2006)	Me-1 6yr (2007)	Bü 8yr (2005)	Ri 6yr (2007)
Phosphonates								
2-AEP	66	56	35	34	24	20	14	15
Other ^a	39	7	26	6	8	4	7	12
Phosphomonoester								
Broad signal	121	64	36	29	47	26	21	24
<i>myo</i> -IP ₆	286	169	192	100	105	54	55	45
<i>scyllo</i> -IP ₆	98	73	37	51	39	39	27	35
<i>neo</i> -IP ₆	21	11	9	7	8	5	4	6
<i>D-chiro</i> -IP ₆	7	5	6	3	2	2	1	2
Other ^a	81	48	82	24	57	22	26	24
Phosphodiester								
DNA	18	11	28	6	24	8	9	13
Other ^a	11	0	9	<1	0	1	0	2
Orthophosphate	5148	2182	1576	932	1341	962	1918	2492
Polyphosphate								
Pyrophosphate	58	22	81	13	40	9	14	14
Other ^a	6	0	1	0	0	0	0	0
Unknown ^b	8	5	2	1	3	1	<1	3
Total P	5968	2653	2121	1207	1698	1153	2098	2686
Inorganic P	5212	2204	1658	945	1380	972	1932	2505
Organic P	756	449	463	262	317	181	166	180
% P _{org} of total	21	27	33	33	29	25	13	11

Abbreviations: CI-LS, clinopyroxene-dominated lava sand; Fluviatile, Fluviatile sand; Ze-LS, zeolite-dominated lava sand.

^aSummation of all sharp signals of unknown identity within the phosphonate, phosphomonoester, phosphodiester or polyphosphate regions.

^bCombined NMR signals located between the phosphonate and phosphomonoester region at δ 11.45–11.35 ppm and between the phosphomonoester and phosphodiester region at δ 2.47–2.22 ppm.

The most abundant pool of Po was that of phosphomonoesters, which comprised on average 80% of the Po in NaOH-EDTA extracts (Table 1). This is consistent with previous studies on mineral soils (McLaren et al., 2020). Concentrations of phosphomonoesters seem to accumulate over time, as we find an increase of total phosphomonoesters (i.e., sum of all phosphomonoester species) from the 8 years Fluv CW (214 mg kg⁻¹) to 16 years of treatment (614 mg kg⁻¹; Table 1).

The main P compounds identified in the phosphomonoester region were *myo*-, *scyllo*-, *neo*- and *D-chiro*-inositol hexakisphosphate (IP₆), and a broad signal (Figure 4 and Table 1). Pools of IP₆ comprised between 47% and 85% of phosphomonoesters across all samples. The *myo* stereoisomer was the most predominant, which comprised on average 64% of total IP₆. *myo*-Inositol hexakisphosphate is an important molecule in living organisms, and particularly in plants, where it functions as a P storage in seeds for the developing seedling. Concentrations of *myo*-IP₆ at the Fluv CW increased from 100 mg kg⁻¹ after 8 years of treatment to 286 mg kg⁻¹ after 16 years of treatment. A

similar increasing trend over time might already be seen at the CI-LS CW and Ze-LS CW, however a 2 years' period is too short to delineate a trend. The accumulation of *myo*-IP₆ in these samples is attributed to the addition of seeds from growing plants, which contain high concentrations of *myo*-IP₆ (Noack et al., 2012), and/or any *myo*-IP₆ potentially present in the wastewater and/or synthesis by microbes. The *myo*-IP₆ can then be stabilized via sorption to metal surfaces (Celi et al., 1999), thus protecting the *myo*-IP₆ from enzymatic hydrolysis (Tang et al., 2006). Concentrations of *scyllo*-IP₆, *neo*-IP₆, *D-chiro*-IP₆ generally followed a similar trend to that of *myo*-IP₆ with operation time across the CW sites, although differences were less pronounced at the CI-LS CW and Ze-LS CW sites. The origin of these IP₆ stereoisomers is unknown, but their abundance is considered to be related to that of *myo*-IP₆ and microbial processes (e.g., epimerization) (Giles et al., 2011; Moata et al., 2016).

Concentrations of the broad signal in the phosphomonoester region ranged from 29 mg kg⁻¹ after 8 years of treatment to 121 mg kg⁻¹ after 16 years of treatment at the Fluv CW (Table 1). A

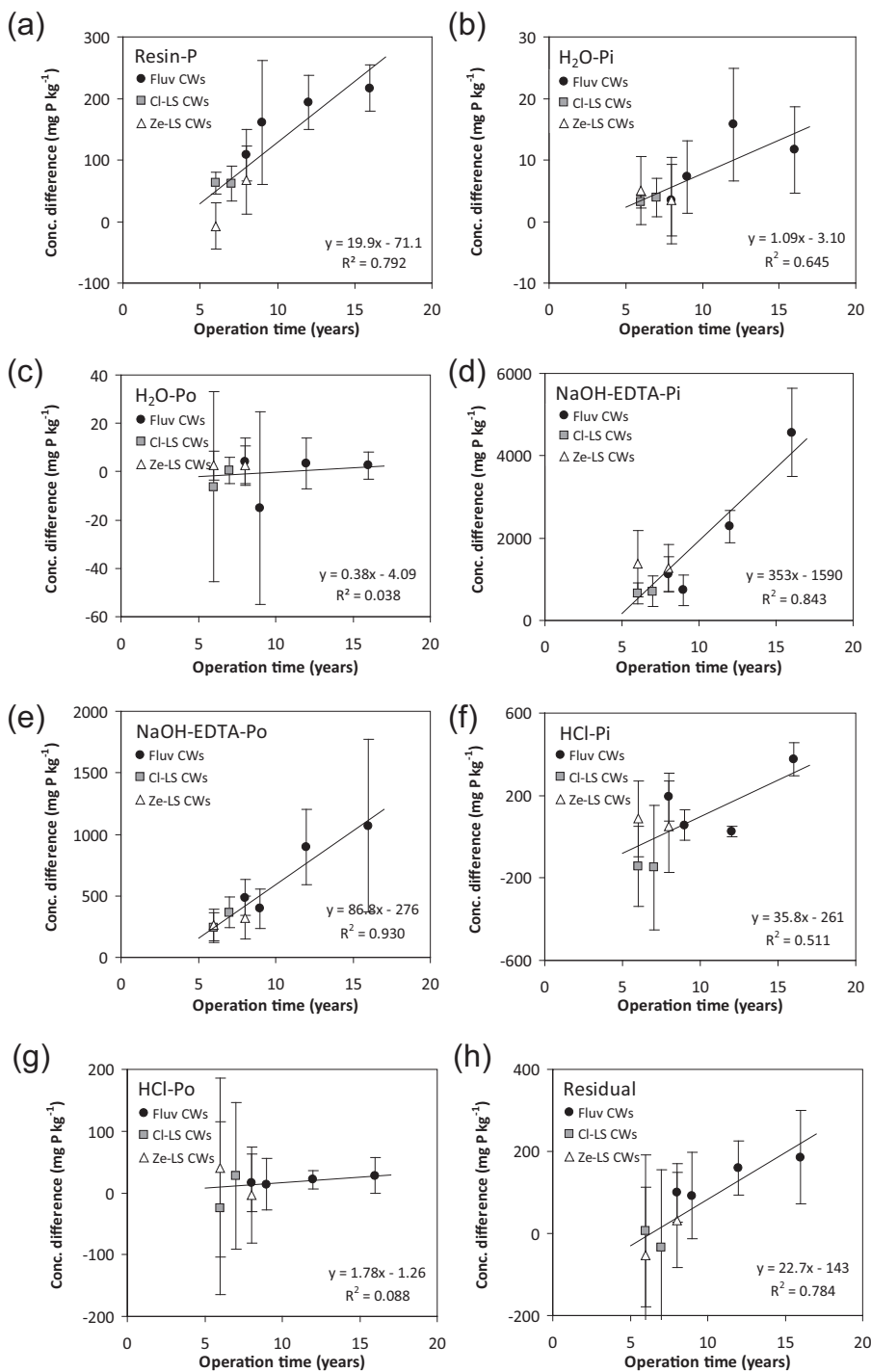


FIGURE 3 Correlation between operation time and concentrations difference between the surface layer (0–10 cm) and the 40–50 cm layer of (a) readily soluble phosphorus (resin-P), (b) water extractable inorganic phosphorus (H₂O-Pi), (c) water extractable organic phosphorus (H₂O-Po), (d) Al and Fe bound inorganic phosphorus (NaOH-EDTA-Pi), (e) Al and Fe bound organic phosphorus (NaOH-EDTA-Po), (f) Ca and Mg bound inorganic phosphorus (HCl-Pi), (g) Ca and Mg bound organic phosphorus (HCl-Po) and (h) residual fraction

similar trend occurred at the CI-LS CW and Ze-LS CW. Phosphomonoesters in the broad signal have been shown to be due to complex forms of Po in high molecular weight material that exist in supra-/macro-molecular structures (Jarosch et al., 2015; McLaren et al., 2015; McLaren et al., 2019). The sequestration of complex forms of Po with operation time across the CW sites is consistent with the accumulation of organic C, and likely involves metals as part of the organic matrix. Whilst the exact mechanisms remain unclear, their sequestration appears to be related to humification processes

(Doolette et al., 2017; Hamdan et al., 2012; Sefe et al., 2015). Here, the accumulation of broad phosphomonoesters is likely to occur via the degradation of plant and/or microbial contents, with a subsequent stabilization reaction with metals and other carbon molecules.

Pools of unknown sharp peaks in the phosphomonoester region (phosphomonoester ‘other’ in Table 1) ranged from 22 to 82 mg kg⁻¹ across all samples, which comprised on average 17% of total phosphomonoesters (Table 1). The identity of these Po species is unknown, but is likely due to a myriad of lower order (IP₅ to IP₁)

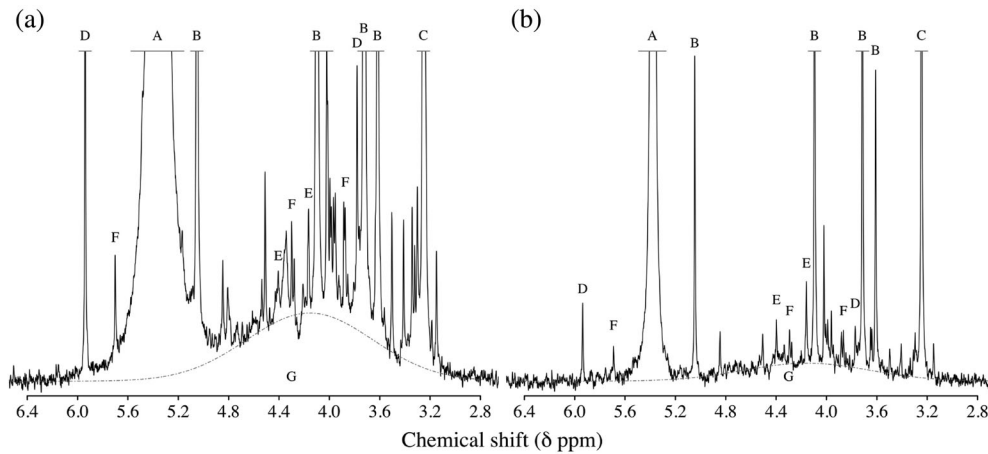


FIGURE 4 Solution ^{31}P nuclear magnetic resonance (NMR) spectra of the orthophosphate and phosphomonoester region (δ 6.4–2.8 ppm) on NaOH-EDTA extracts for the 0–10 cm layer of fluvial sand (Fluv) constructed wetland after (a) 16 years and (b) 8 years operation time. Phosphorus species within the region have been identified as: A – orthophosphate (δ 5.38 ppm), B – *myo*-inositol hexakisphosphate (δ 5.05, 4.10, 3.72 and 3.61 ppm), C – *scyllo*-inositol hexakisphosphate (δ 3.25 ppm), D – *neo*-inositol hexakisphosphate in the 4-equatorial/2-axial position (δ 5.94 and 3.77 ppm), E – *neo*-inositol hexakisphosphate in the 2-equatorial/4-axial position (δ 4.31 and 4.16 ppm), F – *D-chiro*-inositol hexakisphosphate in the 2-equatorial/4-axial position (δ 5.69, 4.29 and 3.87 ppm), and G – broad signal (centred at around δ 4.13 ppm with a line-width of 181 Hz)

inositol phosphates (Reusser et al., 2019). This is because a variety of enzymes are known to dephosphorylate IP_6 along a continuum of lower order inositol phosphates (Cosgrove, 1970; Sun et al., 2017), which could then become stabilized by metal surfaces.

Phosphonates were the second most abundant pool of Po, which comprised on average 13% of the extractable Po across all filter materials (Table 1 and Figure S1 of the Supporting Information). This is higher than typically reported for mineral soils, where phosphodiester are generally the second most abundant pool of Po (Jarosch et al., 2015; McLaren et al., 2015). Increasing concentrations of phosphonates with operation time may be a result of elevated microbial populations under nutrient rich regular addition of organic substances. This pool of Po is likely to be recyclable and bioavailable under different conditions, such as warmer temperatures and less precipitation (Condon et al., 2005).

3.4 | Sorption mechanisms and degree of phosphorus saturation of filter materials

The DPS results were close to or slightly above 100% in all Fluv-CW surface layers, whereas the DPS values decreased to 35%–50% in the 40–50 cm layer (Figure 5a). In CWs with lava sand, DPS values in surface layers (25%–34%) were only slightly higher than in deeper layers (14%–19%), despite significant P accumulation. This suggests a high P sorption capacity at these sites, most likely due to their specific surface areas being a level of magnitude higher than those of Fluv sands. The P saturation in the Fluv-CW surface layer is consistent with their low surface area for P adsorption compared to that of the other filter materials. A similar DPS value for the surface layer of Fluv-16yr (117%) compared to that of the other Fluv CWs (92–123%) was found, although much

more P accumulated in the Fluv-16yr surface layer (Figure 1a, and Table S2 of the Supporting Information). Therefore, it is likely that increasing inputs of P at the Fluv-CWs will accumulate as Po and/or transfer to deeper layers where P saturation has not yet occurred. The correlation of phosphate sorption to oxalate extractable Fe and Al in wetlands is well documented (e.g., Reddy et al., 1995; Richardson, 1985). Our results indicate, that even though Al and Fe(hydr)oxides offer important binding sites for P, they are not the sole regulating mechanisms of P retention in these artificial wetland systems.

High concentrations of oxalate extractable Fe and Al in the 40–50 cm layer of Cl-LS and Ze-LS CWs indicate the high Fe and Al contents in the original substrates. In contrast, concentrations of oxalate extractable Al and Fe were very low in the 40–50 cm layer at Fluv CWs (0.06 g Al kg^{-1} and 0.1 g Fe kg^{-1} , respectively), but accumulated in the 0–10 cm layer. In particular, there was a net accumulation of oxalate extractable Al in the surface layer of Fluv CWs, which increased with operation time and was concomitant with accumulation of P and high DPS values (Figure 6).

Addition of cationic metals such as Fe^{3+} , Al^{3+} and Ca^{2+} is known to enhance the efficiency of P immobilization (Karjalainen et al., 2016; Laakso et al., 2016; Ronkanen et al., 2016). Filter materials amended with different metal salts demonstrated the effectiveness in immobilizing soluble P in CWs in the order of $\text{FeCl}_3 > \text{Al}_2(\text{SO}_4)_3 > \text{Ca}(\text{OH})_2 > \text{CaCO}_3 > \text{CaMg}(\text{CO}_3)_2$ (Ann et al., 2000). Using column leaching experiments, Fe and Al containing inflow water was shown to increase P removal (up to 7 fold) and extend retention capacity (10–100 times) of peatland soils as compared to metal free water (Ronkanen et al., 2016). In addition, the authors reported a strong and significant correlation between removal of P and removal of Fe and Al at this site. Therefore, interaction between P and Al contained in the inflow water may explain the increased accumulation of oxalate

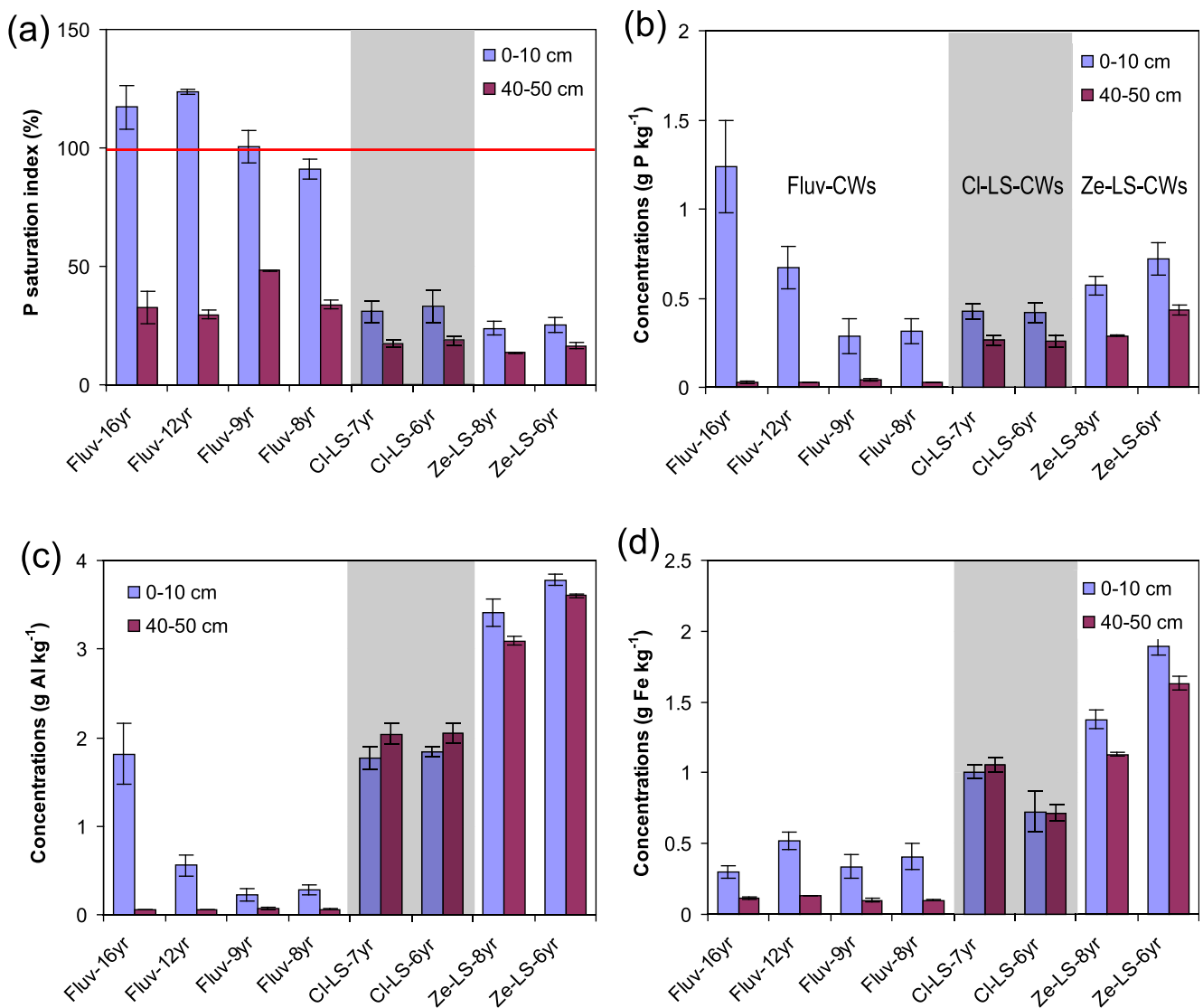


FIGURE 5 (a) Phosphorus saturation index (DPS) and (b) concentrations of oxalate extractable P, (c) Al and (d) Fe at 0–10 and 40–50 cm depths (20–30 cm depth at constructed wetland [CW] Ze-LS-6yr) of CW profiles based on fluvial sand (Fluv), clinopyroxene-dominated lava sand (CI-LS, shaded grey) and zeolite-dominated lava sand (Ze-LS). Standard deviations of three spatial replicates at each site are shown

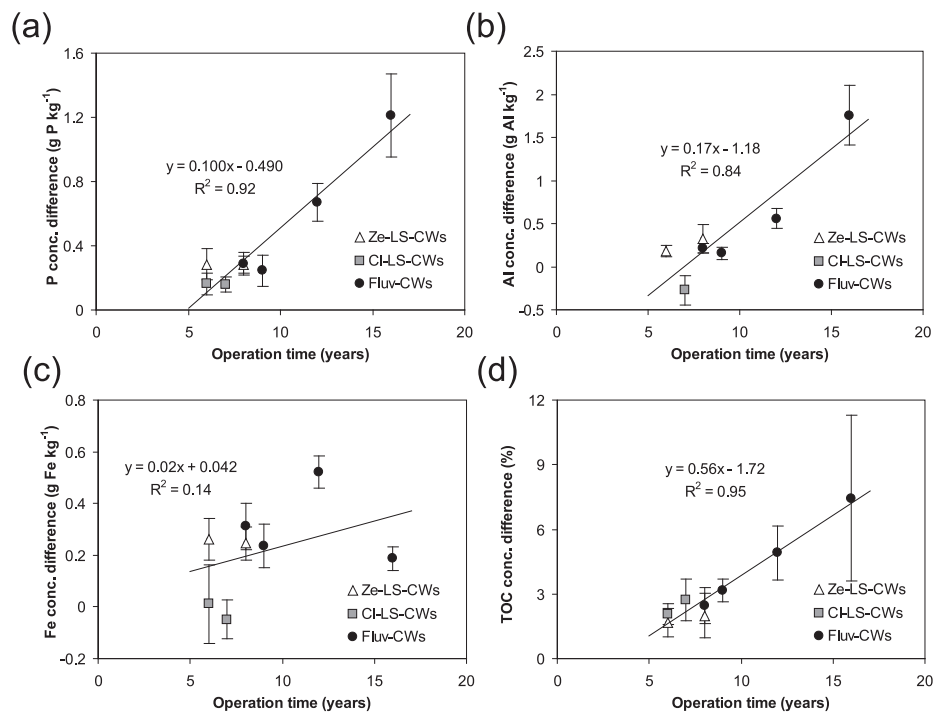
extractable P with operation time at the Fluv CWs, and extended P retention capacity in the surface layers of older Fluv CWs.

There was a strong correlation between concentrations of oxalate extractable P and those of Al and organic C ($r = 0.97$, $p < 0.01$, Table S3 of the Supporting Information), which provide further support for the strong association of Al with P and their retention in CWs. This finding is also consistent with the accumulation of Al and Fe bound P (e.g., NaOH-EDTA extractable P_i and P_o) in all CWs. Orthophosphate has high adsorption affinity to amorphous Al (hydr) oxides and there is the potential of forming secondary Al-P minerals (Ronkanen et al., 2016). Additionally, there was also a strong correlation between concentrations of oxalate extractable P and organic C ($r = 0.90$, $p < 0.01$). These results are consistent with increases in the concentration of P_o over time, and suggest they are closely associated with amorphous metals and organic C.

Interaction between P and Fe seem to be less important due to the reductive dissolution of Fe (hydr)oxides during reducing phases of CW operation, as reflected by decreases in reducible Fe ($NH_2OH-HCl$ extractable) at the Fluv, CI-LS and Ze-LS CWs (Huang et al., 2017). As Al-(hydr)oxides are less subject to reduction during anoxic conditions, P retention with Al might be more stable compared to Fe in these systems.

Both Fe- and Al-mediated P immobilization appeared to be negligible at the CI-LS and Ze-LS CWs, since there was little increase in the concentration of Fe_{ox} and Al_{ox} in surface layers compared to deeper horizons at these sites (Figure 5c,d). Since the specific surface areas of both CI-LS ($18 \text{ m}^2 \text{ g}^{-1}$) and Ze-LS ($72\text{--}80 \text{ m}^2 \text{ g}^{-1}$) were higher than that of Fluv ($1 \text{ m}^2 \text{ g}^{-1}$), P, Fe and Al contained in inflow water may efficiently be retained onto the filter material surface via adsorption. These findings are consistent with the DPS values

FIGURE 6 Correlations between the operation time of constructed wetlands (CWs) and the concentration difference of oxalate extractable Fe, Al, P and organic C at 0–10 cm and 40–50 cm depths (20–30 cm depth at Ze-LS-6 years CW) in all CWs investigated



reported here, which indicated only about 30% P saturation at the time of sampling.

4 | CONCLUSIONS

P accumulation increases with age of the investigated vertical flow CWs in all three investigated filter materials (fluviate sands, zeolite- and clinopyroxene-dominated lava sands). Even though CWs continue to be efficient in accumulating P (and thus removing P from wastewater), the DPS is 100% or even more in the fluviate sand operated CWs. Values greater than 100% DPS indicate that processes other than sorption to Fe and Al play a crucial role in these systems.

The dominant forms of extractable P were poorly crystalline inorganic P (Pi) associated to Fe- and Al (hydr)oxides or to Ca and Mg minerals, with lower contribution of organic P (Po) especially in the fluviate CWs (Fluv CWs). While Po fractions indicate low recycling potential under relatively large scale operating unit conditions, P bound to Fe and Al(hydr)oxides or Ca and Mg minerals might be readily desorbed under anoxic and/or slightly acidic conditions. Solution ³¹P NMR spectroscopy on NaOH-EDTA extracts of filter materials indicated a large portion of the organic P were pools of IP₆, and 'complex' phosphomonoesters (i.e., broad signal) that is associated with large molecular weight material and humic substances. These pools were also important sinks of wastewater P over time. While a retention of P in organic forms might indicate a potentially unlimited sequestration potential, it simultaneously restricts the recycling (e.g., desorption) capacity of P from these materials.

As we investigated vertical flow CWs, most of the phosphorous is accumulated in the upper horizons. Future research should concentrate on desorption/extraction experiments or on experiments using the filter material of the upper horizons directly as fertilizer, as our

results indicate high bioavailability of the major P pools. However, maximum substrate contents of Al and Fe bound inorganic P of 0.5% in the surface horizon of the oldest fluviate and up to 0.4% in the zeolite dominated lava sands are still too low for direct fertilizer use.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Alewell, C., Ringeval, B., Ballabio, C., Robinson, D. A., Panagos, P., & Borrelli, P. (2020). Global phosphorus shortage will be aggravated by soil erosion. *Nature Communications*, 11(1), 4546. <https://doi.org/10.1038/s41467-020-18326-7>
- Anderson, J. M., & Ingram, J. S. I. (1993). *Tropical soil biology and fertility: A handbook of methods* (pp. 70–71). Wallingford, Oxfordshire: CAB International (1993).

- Ann, Y., Reddy, K. R., & Delfino, J. J. (2000). Influence of chemical amendments on phosphorus immobilization in soils from a constructed wetland. *Ecological Engineering*, 14(1–2), 157–167.
- Ballantine, D. J., & Tanner, C. C. (2010). Substrate and filter materials to enhance phosphorus removal in constructed wetlands treating diffuse farm runoff: A review. *New Zealand Journal of Agricultural Research*, 53(1), 71–95. <https://doi.org/10.1080/00288231003685843>
- Bowman, R. A., & Moir, J. O. (1993). Basic EDTA as an extractant for soil organic phosphorus. *Soil Science Society of America Journal*, 57(6), 1516–1518.
- Bruch, I., Alewell, U., Hahn, A., Hasselbach, R., & Alewell, C. (2014). Influence of soil physical parameters on removal efficiency and hydraulic conductivity of vertical flow constructed wetlands. *Ecological Engineering*, 68, 124–132. <https://doi.org/10.1016/j.ecoleng.2014.03.069>
- Bruch, I., Alewell, U., & Hasselbach, R. (2011a). *Reinigungsprozess in Lavasand-Bodenfiltern in der Mischwasserbehandlung: 2. Stufe: Dokumentation der mikrobiologischen und substratabhängigen Reinigungsprozesse in Abhängigkeit von der Dauer des Betriebs (Kläranlage Riesweiler)*. Retrieved from <https://www.dr-bruch-und-partner.de>
- Bruch, I., Fritsche, J., Banninger, D., Alewell, U., Sendelov, M., Huerlimann, H., ... Alewell, C. (2011b). Improving the treatment efficiency of constructed wetlands with zeolite-containing filter sands. *Bioresource Technology*, 102(2), 937–941. <https://doi.org/10.1016/j.biortech.2010.09.041>
- Cade-Menun, B. J., & Preston, C. M. (1996). A comparison of soil extraction procedures for P-31 NMR spectroscopy. *Soil Science*, 161(11), 770–785. <https://doi.org/10.1097/00010694-199611000-00006>
- Celi, L., Lamacchia, S., Marsan, F. A., & Barberis, E. (1999). Interaction of inositol hexaphosphate on clays: Adsorption and charging phenomena. *Soil Science*, 164(8), 574–585. <https://doi.org/10.1097/00010694-199908000-00005>
- Condron, L. M., Turner, B. L., & Cade-Menun, B. J. (2005). Chemistry and dynamics of soil organic phosphorus. In J. T. Sims, A. N. Sharpley & D. T. Westermann (Eds.), *Phosphorus: Agriculture and the Environment*, 87–121. <https://doi.org/10.2134/agronmonogr46.c4>
- Cosgrove, D. J. (1970). Inositol phosphate phosphatases of microbiological origin. Inositol phosphate intermediates in the dephosphorylation of the hexaphosphates of myo-inositol, scyllo-inositol, and D-chiro-inositol by a bacterial (*Pseudomonas* sp.) phytase. *Australian Journal of Biological Sciences*, 23(5), 1207–1220. <https://doi.org/10.1071/bi9701207>
- Cui, L. H., Zhu, X. Z., Ma, M., Ouyang, Y., Dong, M., Zhu, W. L., & Luo, S. M. (2008). Phosphorus sorption capacities and physicochemical properties of nine substrate materials for constructed wetland. *Archives of Environmental Contamination and Toxicology*, 55(2), 210–217. <https://doi.org/10.1007/s00244-007-9109-y>
- Daly, K., Jeffrey, D., & Tunney, H. (2001). The effect of soil type on phosphorus sorption capacity and desorption dynamics in Irish grassland soils. *Soil Use and Management*, 17(1), 12–20.
- Davis, S. J., hUallachain, D. O., Mellander, P. E., Matthaei, C. D., Piggott, J. J., & Kelly-Quinn, M. (2019). Chronic nutrient inputs affect stream macroinvertebrate communities more than acute inputs: An experiment manipulating phosphorus, nitrogen and sediment. *Science of the Total Environment*, 683, 9–20. <https://doi.org/10.1016/j.scitotenv.2019.05.031>
- Doolette, A. L., Smernik, R. J., & Dougherty, W. J. (2010). Rapid decomposition of phytate applied to a calcareous soil demonstrated by a solution ³¹P NMR study. *European Journal of Soil Science*, 61(4), 563–575. <https://doi.org/10.1111/j.1365-2389.2010.01259.x>
- Doolette, A. L., Smernik, R. J., & McLaren, T. I. (2017). The composition of organic phosphorus in soils of the Snowy Mountains region of south-eastern Australia. *Soil Research*, 55(1), 10–18. <https://doi.org/10.1071/sr16058>
- Gerke, J. (2010). Humic (organic matter)-Al(Fe)-phosphate complexes: An underestimated phosphate form in soils and source of plant-available phosphate. *Soil Science*, 175(9), 417–425. <https://doi.org/10.1097/SS.0b013e3181f1b4dd>
- Giles, C. D., Cade-Menun, B. J., & Hill, J. E. (2011). The inositol phosphates in soils and manures: Abundance, cycling, and measurement. *Canadian Journal of Soil Science*, 91(3), 397–416. <https://doi.org/10.4141/cjss09090>
- Guitttony-Philippe, A., Masotti, V., Hohener, P., Boudenne, J. L., Viglione, J., & Laffont-Schwob, I. (2014). Constructed wetlands to reduce metal pollution from industrial catchments in aquatic Mediterranean ecosystems: A review to overcome obstacles and suggest potential solutions. *Environment International*, 64, 1–16. <https://doi.org/10.1016/j.envint.2013.11.016>
- Guppy, C. N., Menzies, N. W., Moody, P. W., & Blamey, F. P. C. (2005). Competitive sorption reactions between phosphorus and organic matter in soil: A review. *Australian Journal of Soil Research*, 43(2), 189–202. <https://doi.org/10.1071/sr04049>
- Hamdan, R., El-Rifai, H. M., Cheesman, A. W., Turner, B. L., Reddy, K. R., & Cooper, W. T. (2012). Linking phosphorus sequestration to carbon humification in wetland soils by ³¹P and ¹³C NMR spectroscopy. *Environmental Science & Technology*, 46(9), 4775–4782. <https://doi.org/10.1021/es204072k>
- Hasselbach, R. (2013). *Leistungsfähigkeit von Pflanzenkläranlagen im Mischsystem mit Lavasand als Filtersubstrat*. (PhD), Technischen Universität Kaiserslautern, Kaiserslautern. pp. D380.
- Huang, J. H., Paul, S., Mayer, S., Moradpour, E., Hasselbach, R., Giere, R., & Alewell, C. (2017). Metal biogeochemistry in constructed wetlands based on fluvial sand and zeolite- and clinopyroxene-dominated lava sand. *Scientific Reports*, 7, 2981. <https://doi.org/10.1038/s41598-017-03055-7>
- Huber, L. (2012). *Phosphor in Pflanzenkläranlagen - Konzentrationen und Bindungsformen*. (Masterthesis), 1–81. Basel, Switzerland: Universität Basel.
- Jarosch, K. A., Doolette, A. L., Smernik, R. J., Tamburini, F., Frossard, E., & Bunemann, E. K. (2015). Characterisation of soil organic phosphorus in NaOH-EDTA extracts: A comparison of P-31 NMR spectroscopy and enzyme addition assays. *Soil Biology & Biochemistry*, 91, 298–309. <https://doi.org/10.1016/j.soilbio.2015.09.010>
- Karjalainen, S. M., Ronkanen, A. K., Heikkinen, K., & Klove, B. (2016). Long-term accumulation and retention of Al, Fe and P in peat soils of northern treatment wetlands. *Ecological Engineering*, 93, 91–103. <https://doi.org/10.1016/j.ecoleng.2016.05.004>
- Kuusemets, V., & Lohmus, K. (2005). Nitrogen and phosphorus accumulation and biomass production by *Scirpus sylvaticus* and *Phragmites australis* in a horizontal subsurface flow constructed wetland. *Journal of Environmental Science and Health Part A - Toxic/Hazardous Substances & Environmental Engineering*, 40(6–7), 1167–1175. <https://doi.org/10.1081/ese-200055629>
- Laakso, J., Uusitalo, R., & Yli-Halla, M. (2016). Phosphorus speciation in agricultural catchment soils and in fresh and dried sediments of five constructed wetlands. *Geoderma*, 271, 18–26. <https://doi.org/10.1016/j.geoderma.2016.02.007>
- Lind, L., Hasselquist, E. M., & Laudon, H. (2019). Towards ecologically functional riparian zones: A meta-analysis to develop guidelines for protecting ecosystem functions and biodiversity in agricultural landscapes. *Journal of Environmental Management*, 249, 109391. <https://doi.org/10.1016/j.jenvman.2019.109391>
- Lü, C. W., Yan, D. H., He, J., Zhou, B., Li, L., & Zheng, Q. (2017). Environmental geochemistry significance of organic phosphorus: An insight from its adsorption on iron oxides. *Applied Geochemistry*, 84, 52–60. <https://doi.org/10.1016/j.apgeochem.2017.05.026>
- McLaren, T. I., Smernik, R. J., McLaughlin, M. J., Doolette, A. L., Richardson, A. E., & Frossard, E. (2020). The chemical nature of soil

- organic phosphorus: A critical review and global compilation of quantitative data. In D. L. Sparks (Ed.), *Advances in agronomy* (Vol. 160, pp. 51–124). Academic Press.
- McLaren, T. I., Smernik, R. J., McLaughlin, M. J., McBeath, T. M., Kirby, J. K., Simpson, R. J., ... Richardson, A. E. (2015). Complex forms of soil organic phosphorus – A major component of soil phosphorus. *Environmental Science & Technology*, 49(22), 13238–13245. <https://doi.org/10.1021/acs.est.5b02948>
- McLaren, T. I., Verel, R., & Frossard, E. (2019). The structural composition of soil phosphomonoesters as determined by solution ^{31}P NMR spectroscopy and transverse relaxation (T2) experiments. *Geoderma*, 345, 31–37. <https://doi.org/10.1016/j.geoderma.2019.03.015>
- Moata, M. R. S., Doolette, A. L., Smernik, R. J., McNeill, A. M., & Macdonald, L. M. (2016). Organic phosphorus speciation in Australian Red Chromosols: Stoichiometric control. *Soil Research*, 54(1), 11–19. <https://doi.org/10.1071/sr15085>
- Moradpour, E., & Mayer, S. (2012). *Accumulation of C, N, P and selected heavy metals in constructed wetlands for wastewater treatment in Rheinland-Pfalz and Saarland, Germany* (Master's thesis). University of Basel, Basel.
- Nair, V. D., Portier, K. M., Graetz, D. A., & Walker, M. L. (2004). An environmental threshold for degree of phosphorus saturation in sandy soils. *Journal of Environmental Quality*, 33(1), 107–113.
- Noack, S. R., McLaughlin, M. J., Smernik, R. J., McBeath, T. M., & Armstrong, R. D. (2012). Crop residue phosphorus: Speciation and potential bio-availability. *Plant and Soil*, 359(1–2), 375–385. <https://doi.org/10.1007/s11104-012-1216-5>
- Ohno, T., & Zibilske, L. M. (1991). Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Science Society of America Journal*, 55(3), 892–895. <https://doi.org/10.2136/sssaj1991.03615995005500030046x>
- Olila, O. G., Reddy, K. R., & Stites, D. L. (1997). Influence of draining on soil phosphorus forms and distribution in a constructed wetland. *Ecological Engineering*, 9(3–4), 157–169. [https://doi.org/10.1016/s0925-8574\(97\)10006-4](https://doi.org/10.1016/s0925-8574(97)10006-4)
- Paludan, C., & Jensen, H. S. (1995). Sequential extraction of phosphorus in freshwater wetland and lake sediment: Significance of humic acids. *Wetlands*, 15(4), 365–373. <https://doi.org/10.1007/bf03160891>
- Pant, H. K. (2020). Estimation of internal loading of phosphorus in freshwater wetlands. *Current Pollution Reports*, 6(1), 28–35. <https://doi.org/10.1007/s40726-020-00136-6>
- Paytan, A., & McLaughlin, K. (2007). The oceanic phosphorus cycle. *Chemical Reviews*, 107(2), 563–576. <https://doi.org/10.1021/cr0503613>
- R_Core_Team. (2016). *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://cran.r-project.org/doc/manuals/r-release/fullrefman.pdf>
- Reddy, K. R., Diaz, O. A., Scinto, L. J., & Agami, M. (1995). Phosphorus dynamics in selected wetlands and streams of the Lake Okeechobee Basin. *Ecological Engineering*, 5(2–3), 183–207. [https://doi.org/10.1016/0925-8574\(95\)00024-0](https://doi.org/10.1016/0925-8574(95)00024-0)
- Reusser, J. E., Verel, R., Frossard, E., & McLaren, T. I. (2020). Quantitative measures of myo-IP6 in soil using solution ^{31}P NMR spectroscopy and spectral deconvolution fitting including a broad signal. *Environmental Science: Processes & Impacts*, 22(4), 1084–1094. <https://doi.org/10.1039/C9EM00485H>
- Reusser, J. E., Verel, R., Zindel, D., Frossard, E., & McLaren, T. I. (2019). Identification of lower-order inositol phosphates (IP5 and IP4) in soil extracts as determined by hypobromite oxidation and solution ^{31}P NMR spectroscopy. *Biogeosciences Discussions*, 2019, 1–28. <https://doi.org/10.5194/bg-2019-431>
- Richardson, C. J. (1985). Mechanisms controlling phosphorus retention capacity in fresh-water wetlands. *Science*, 228(4706), 1424–1427. <https://doi.org/10.1126/science.228.4706.1424>
- Ronkanen, A. K., Marttila, H., Celebi, A., & Klove, B. (2016). The role of aluminium and iron in phosphorus removal by treatment peatlands. *Ecological Engineering*, 86, 190–201. <https://doi.org/10.1016/j.ecoleng.2015.11.011>
- Scheffe, C. R., Barlow, K. M., Robinson, N. J., Crawford, D. M., McLaren, T. I., Smernik, R. J., ... Kitching, M. (2015). 100 Years of superphosphate addition to pasture in an acid soil-current nutrient status and future management. *Soil Research*, 53(6), 662–676. <https://doi.org/10.1071/sr14241>
- Schoumans, O. F. (2000). Determination of the degree of phosphate saturation in non-calcerous soils. In G. M. Pierzynski (Ed.), *Methods of phosphorus analysis for soils, sediments, residuals, and waters* (pp. 31–34). North Carolina State University.
- Singh, B., & Gilkes, R. J. (1991). Phosphorus sorption in relation to soil properties for the major soil types of south-western Australia. *Australian Journal of Soil Research*, 29(5), 603–618. <https://doi.org/10.1071/sr9910603>
- Sohrt, J., Lang, F., & Weiler, M. (2017). Quantifying components of the phosphorus cycle in temperate forests. *Wiley Interdisciplinary Reviews: Water*, 4(6), e1243. <https://doi.org/10.1002/wat2.1243>
- Sun, M. J., Alikhani, J., Massoudieh, A., Greiner, R., & Jaisi, D. P. (2017). Phytate degradation by different phosphohydrolase enzymes: contrasting kinetics, decay rates, pathways, and isotope effects. *Soil Science Society of America Journal*, 81(1), 61–75. <https://doi.org/10.2136/sssaj2016.07.0219>
- Tang, J., Leung, A., Leung, C., & Lim, B. L. (2006). Hydrolysis of precipitated phytate by three distinct families of phytases. *Soil Biology & Biochemistry*, 38(6), 1316–1324. <https://doi.org/10.1016/j.soilbio.2005.08.021>
- Tiessen, H., & Moir, J. O. (1993). Characterization of available P by sequential extraction. In M. R. Carter (Ed.), *Soil sampling and methods of analysis* (pp. 75–86). Boca Raton, FL: Lewis.
- Turner, B. L., Newman, S., & Newman, J. M. (2006a). Organic phosphorus sequestration in subtropical treatment wetlands. *Environmental Science & Technology*, 40(3), 727–733. <https://doi.org/10.1021/es0516256>
- Turner, B. L., Newman, S., & Reddy, K. R. (2006b). Overestimation of organic phosphorus in wetland soils by alkaline extraction and molybdate colorimetry. *Environmental Science & Technology*, 40(10), 3349–3354. <https://doi.org/10.1021/es052442m>
- Vera, I., Araya, F., Andres, E., Saez, K., & Vidal, G. (2014). Enhanced phosphorus removal from sewage in mesocosm-scale constructed wetland using zeolite as medium and artificial aeration. *Environmental Technology*, 35(13), 1639–1649. <https://doi.org/10.1080/09593330.2013.877984>
- Vohla, C., Koiv, M., Bavor, H. J., Chazarenc, F., & Mander, U. (2011). Filter materials for phosphorus removal from wastewater in treatment wetlands – A review. *Ecological Engineering*, 37(1), 70–89. <https://doi.org/10.1016/j.ecoleng.2009.08.003>
- Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment*, 380(1–3), 48–65. <https://doi.org/10.1016/j.scitotenv.2006.09.014>
- Withers, P. J. A., & Jarvie, H. P. (2008). Delivery and cycling of phosphorus in rivers: A review. *Science of the Total Environment*, 400(1–3), 379–395. <https://doi.org/10.1016/j.scitotenv.2008.08.002>
- Wu, S. B., Lyu, T., Zhao, Y. Q., Vymazal, J., Arias, C. A., & Brix, H. (2018). Rethinking intensification of constructed wetlands as a green eco-technology for wastewater treatment. *Environmental Science & Technology*, 52(4), 1693–1694. <https://doi.org/10.1021/acs.est.8b00010>
- Xu, D. F., Xu, J. M., Wu, J. J., & Muhammad, A. (2006). Studies on the phosphorus sorption capacity of substrates used in constructed wetland systems. *Chemosphere*, 63(2), 344–352. <https://doi.org/10.1016/j.chemosphere.2005.08.036>
- Zhang, L., Lyu, T., Vargas, C. A. R., Arias, C. A., Carvalho, P. N., & Brix, H. (2018). New insights into the effects of support matrix on the removal

of organic micro-pollutants and the microbial community in constructed wetlands. *Environmental Pollution*, 240, 699–708. <https://doi.org/10.1016/j.envpol.2018.05.028>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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